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Relationship Between Hairy Cell Leukemia Variant and Splenic Lymphoma With Villous Lymphocytes: Presentation of a New Concept

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An unusual case of low-grade B-cell lymphoproliferative disorder with peripheral lymphocytosis and splenomegaly followed for 4½ years is reported. During this period, the phenotype of the tumor cells in the blood changed from that of hairy cell leukemia (HCL)/chronic lymphocytic leukemia (CLL) to HCL/prolymphocytic leukemia (PLL), to PLL. The lymphoid population in the blood showed a mixture of hairy cells, villous lymphocytes, small lymphocytes, and prolymphocytes, corresponding to the phenotypes at various stages. Although relatively specific markers for CLL, HCL, and PLL, such as CD5, CD11c, CD22, CD25, and FMC-7, were positive at various stages, all these markers have also been demonstrated in a large study series of splenic lymphoma with villous lymphocytes (SLVL). In addition, the histologic pattern of the bone marrow biopsy and splenectomy specimen were not typical for HCL. This case can therefore be classified either as HCL variant or as SLVL. As SLVL assumes various cytologic and histologic patterns, which overlap with different lymphoproliferative disorders, especially HCL variants, this entity appears to represent a heterogeneous group of lymphomas/leukemias that may evolve into each other. The absence of activation of c-*myc* and *bcl*-2 oncogenes as well as mutation of p53 tumor suppressor gene, together with the presence of only one single rearranged band for both heavy chain and κ light chain genes in our case suggest that these morphologically different lymphoid tumors may belong to the same family.

Key words: hairy cell leukemia, splenic lymphoma with villous lymphocytes, immunophenotyping, molecular biology, subclassification

INTRODUCTION

Splenic lymphoma with villous lymphocytes (SLVL) is a newly described entity similar to hairy cell leukemia (HCL) with differences in histologic patterns and surface markers [1,2]. However, several studies have demonstrated morphologic heterogeneity in SLVL cases. The cytology can be of the small cell, large cell, or mixed small and large cell type [3–6]. A recent study further shows that SLVL cells may react to practically all surface markers currently used for the differential diagnosis of low-grade B-cell lymphomas/leukemias [6]. For instance, CD5, C23, CD10, CD25, and FMC-7, which have been considered relatively specific for the diagnosis of chronic lymphocytic leukemia (CLL)/small lymphocytic lymphoma, follicular lymphoma, HCL, and prolymphocytic leukemia (PLL), respectively, are all reactive to certain percentages of SLVL cases [7]. These studies raise the question as to whether SLVL is a distinct clinicopathologic entity or represents a heterogeneous group of lymphomas/leukemias lumped together into a single disorder.

We report a case of low-grade lymphoproliferative disorder that showed evolution of the lymphoid cell population and phenotype in the peripheral blood over a 4-

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year course. This case suggests that SLVL is probably transformable with other low-grade lymphomas/leukemias or consists of variants of various lymphoproliferative disorders. Further subclassifications of SLVL may be useful for more specific treatment.

CASE REPORT

A 63-year-old woman presented in September 1990 with splenomegaly found on routine examination by her family physician. There was no significant past history. She had noted some slight weight loss but denied fever, night sweats, or itching.

On physical examination, her spleen was felt 9 cm below the left costal margin, measuring about 7 cm across. The liver was felt 4 cm below the right costal margin. There was no lymphadenopathy.

Initial laboratory studies showed a normal hemoglobin, hematocrit, platelet count, erythrocyte sedimentation rate, and reticulocyte count. The white blood cell (WBC) count was 22.5×10^9/L with 79% lymphoid cells, many of which appeared atypical. Some of these lymphocytes had cytoplasmic projections; thus, hairy cell leukemia was suspected. Some smudge cells were also noted. A coagulation screening, urinalysis, and routine chemistries were normal. Immunoglobulin studies showed a mild polyclonal increase in IgM. Bone marrow studies confirmed the diagnosis of lymphoproliferative disorder.

A chest radiograph was normal. A computed tomography (CT) scan of the abdomen confirmed splenomegaly with a suggestion of some enlarged lymph nodes in the region of the left gastric group, but no retroperitoneal lymphadenopathy was seen.

No treatment was instituted. One year later, the spleen was felt 15 cm below the costal margin, measuring 11 cm across. The WBC count had increased to 37×10^9/L, but the patient remained asymptomatic. Two years after initial diagnosis, the spleen measured 20 cm down and 17 cm across, with an essentially unchanged leukocyte count. One year later, the spleen was felt 25 cm below the costal margin and 20 cm across. A repeat CT scan showed massive splenomegaly with compression and displacement of the intra-abdominal organs.

The patient rejected any form of chemotherapy. Therefore, an elective splenectomy was done in May 1994. Following the splenectomy, the leukocyte count rose to 60×10^9/L, and the platelet count to $>1{,}100 \times 10^9$/L. The hemoglobin improved to 12.8 g% with a hematocrit of 39.2%. The leukocyte count gradually decreased but remained elevated with an absolute increase in lymphoid cells. The most recent platelet count (June 1995) was 495×10^9/L. The leukocyte count was 18.9×10^9/L with 74% lymphoid cells. There was clinical improvement after splenectomy, and the patient continued to feel well without additional therapy.

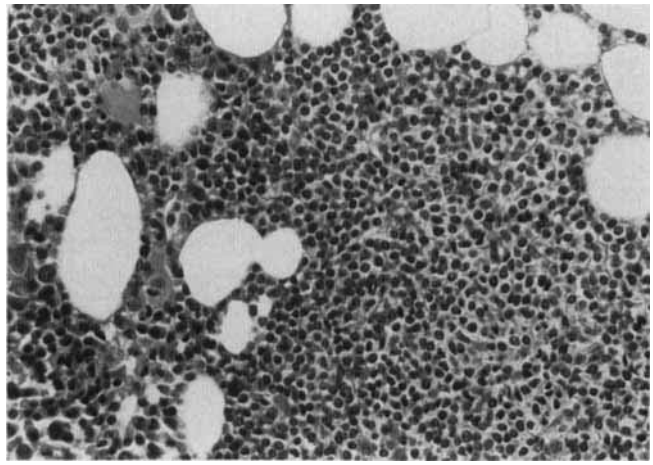

Fig. 1. Bone marrow biopsy showing one of several nonparatrabecular lymphoid aggregates. Some lymphoid cells at the right upper corner are widely spaced mimicking a fried-egg pattern. (Hematoxylin & eosin stain, ×400.)

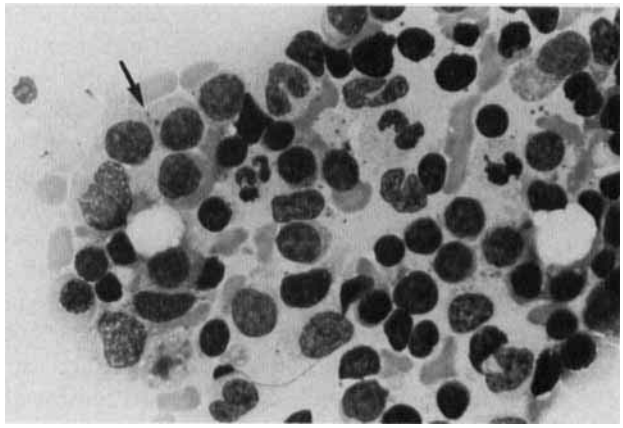

Fig. 2. Bone marrow aspirate showing a few lymphoid cells (arrow) appearing to be hairy cells. The remaining cells are mostly small mature lymphocytes. (Wright-Giemsa stain, ×1,000.)

MATERIALS AND RESULTS

Bone Marrow and Peripheral Blood Smears

The bone marrow biopsy obtained on September 5, 1990 revealed a nodular infiltrative pattern consisting of small mature-looking lymphocytes. A few small foci of widely spaced lymphoid cells (fried-egg pattern) were also noted (Fig. 1). The bone marrow aspirate obtained at the same time showed 45% lymphoid cells with sheets of mature-appearing lymphocytes. A few clusters of medium-size lymphocytes with moderate amount of cytoplasm were also present (Fig. 2). The bone marrow aspi-

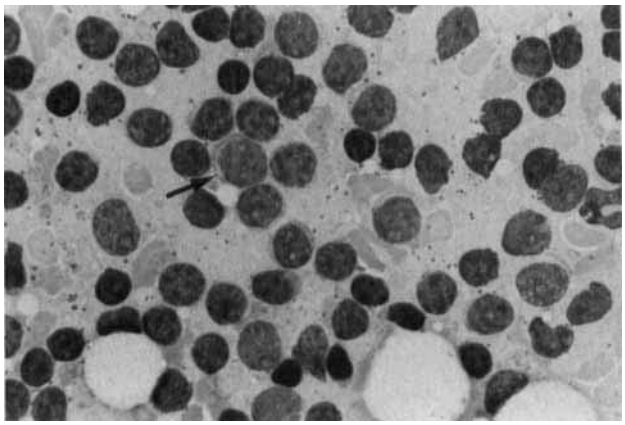

Fig. 3. Bone marrow aspirate on a later date showing mostly prolymphocytes (arrow). (Wright-Giemsa stain, ×1,000.)

rate taken February 28, 1994 revealed 86% lymphoid cells. Most of these were prolymphocytes (Fig. 3).

The peripheral blood smears from June 29, 1992 showed 79% lymphoid cells, about one-third of which had cytoplasmic projections of varying length; some looked like typical hairy cells and others appeared like villous lymphocytes (Fig. 4a,b). The nonvillous lymphoid cells were mostly small lymphocytes with a small population of prolymphocytes. In the following peripheral blood smears obtained in 1993 and 1994, there was a gradual increase in prolymphocytes (Fig. 4c) and a decrease in small lymphocytes. Small percentages of hairy cells and villous lymphocytes were persistently present. After splenectomy, prolymphocytes became predominant, and hairy cells and villous lymphocytes were only occasionally seen. There was also an increase in monocytes.

Electron Microscopy

Electron microscopy examination of a buffy coat preparation from a post-splenectomy specimen demonstrated many villous lymphoid cells. These neoplastic lymphoid cells showed focal distribution of relatively short cytoplasmic projections (Fig. 5). The number of projections per cell ranged from 8 to 12. The cytoplasm was relatively undifferentiated with many free ribosomes, a few strands of endoplasmic reticulum, and scattered mitochondria. Nuclei were round or slightly irregular in contour and showed peripheral margination of heterochromatin, typical of lymphoid cells.

Histopathologic Findings

The splenectomy specimen weighed 3,270 g, measuring 20 × 18 × 12 cm. Cross sections of the spleen showed prominence of the white pulp. Microscopic examination showed expansion of the white pulp and extensive lymphoid cells infiltration in the red pulp cords and sinuses (Figs. 6, 7). The lymphoid cells were mostly medium-size lymphocytes with a moderate amount of cytoplasm. The nuclei showed a clumped chromatin pattern and occasional nucleoli. No prominent blood-lake features were noted. These features were more consistent with SLVL than with HCL.

Immunohistochemistry

The lymphoid cells in the splenectomy specimen were stained predominantly with CD20, CD45, and CD74 antibodies, while negative results were demonstrated with CD3, CD45RO, CDw75, κ, and λ antibodies.

Flow Cytometry

The bone marrow specimen performed September 5, 1990 showed a monoclonal IgM-κ surface immunoglobulin pattern (dim) with 81% CD5, CD11c, CD19, CD22-positive population (Table I). This population was also reactive to tartrate-resistant acid phosphatase. Therefore, the neoplastic population was considered a hybrid form of HCL/CLL. The study on a peripheral blood specimen June 29, 1992 showed essentially the same findings, but the percentage of the tumor population was 76%. In addition, this population also showed 75% CD25-positive cells. The second bone marrow (February 28, 1994) revealed similar findings as previous specimens, but CD25 became negative. By contrast, FMC-7 was found to be positive in 88% of CD5, CD11c, CD22-positive cells. Because of the high percentage of FMC-7-positive population and the presence of large numbers of prolymphocytes, the interpretation was that of a hybrid form of HCL/PLL. The splenectomy specimen (May 9, 1994) showed essentially the same findings as in the second bone marrow, but the CD5, CD11c, CD22, FMC-7-positive population was then 65% and the tartrate-resistant acid phosphatase reaction became negative.

Molecular Biological Studies

Southern blot analysis of the genomic DNA from the spleen using probes from the J-region and S-region of the Ig heavy chain and from the J-region of κ light chain identified clonally rearranged DNA bands in these genes. However, only one rearranged DNA band was seen in all the restriction enzyme digestions. These results indicate the absence of additional DNA changes in the Ig genes accompanying the progression of the disease. Southern blot analysis using probes for *bcl*-2 (MBCR) and c-*myc* (exon 1) genes did not identify rearrangements in these genes. Single-strand confirmation polymorphism (SSCP) analysis of p53 gene exon 5–9 showed no evidence of p53 mutation (Fig. 8).

Cytogenetic Studies

Cytogenetic studies of the lymphocytes from the spleen specimen showed the presence of a clonal population of

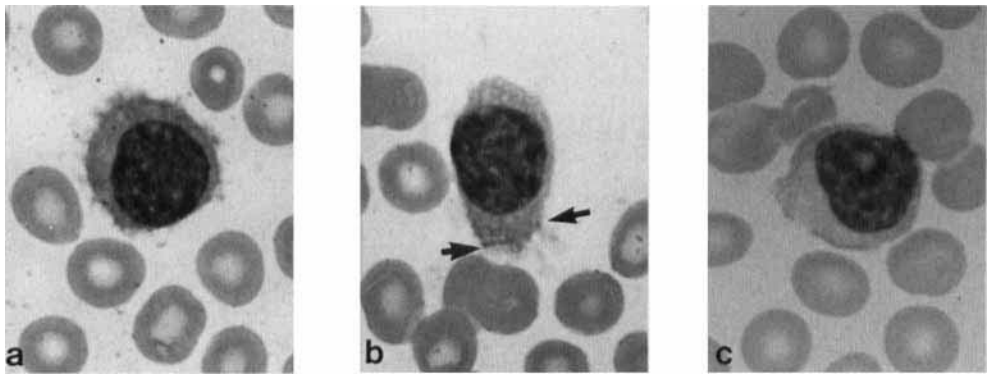

Fig. 4. a: Hairy cell showing a round nucleus, moderate amount of cytoplasm, and numerous cytoplasmic projections surrounding the entire cell. (Wright-Giemsa stain, ×1,000.) b: Villous lymphocyte showing polar distribution of short villi (arrows), less cytoplasm, and more dense chromatin than hairy cell. (Wright-Giemsa stain, ×1,000.) c: Prolymphocyte showing abundant cytoplasm and a prominent nucleolus. (Wright-Giemsa stain, ×1,000.)

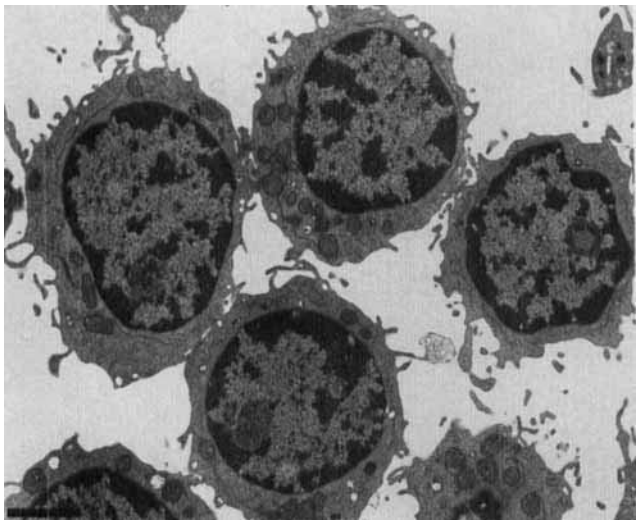

Fig. 5. Electron micrograph of four villous lymphocytes showing focal distribution of relatively short villi. Bar = 2 μm. ×9,300.

cells carrying 46,XX,del(7) (q22q32) in 87% of metaphases.

DISCUSSION

SLVL was described in the earlier literature under various names, such as splenomegalic immunocytoma [5,8], malignant lymphoma simulating leukemic reticuloendotheliosis [3], lymphocytic lymphoma simulating HCL [4], and chronic lymphoproliferative disorder resembling HCL [9]. It was in 1987 that Melo et al. [1,2] first applied the term SLVL to define this entity. This terminology was promptly adopted by the French–American–British (FAB) Cooperative Group in 1989 [10].

The major differential diagnosis for SLVL is HCl and its variants. SLVL and HCL share the common features of splenomegaly and the presence of cytoplasmic projections on the tumor cells. The major difference is their histologic patterns; the typical histologic patterns of HCL, such as pseudosinus formation in the spleen and the fried-egg pattern in the bone marrow have not been reported in SLVL. The white pulp is usually atrophic in HCL but is infiltrated by tumor cells in SLVL.

Without histologic examination, a preliminary diagnosis can be made by peripheral blood examination [1]. SLVL usually shows mild peripheral lymphocytosis with a leukocyte count of 3–38 × 10^9/L. HCL is characterized by pancytopenia, but leukocytosis can be seen in a small percentage of HCL and is frequently demonstrated in its variants [11]. A typical villous lymphocyte is larger than the small lymphocytes and close in size to a prolymphocyte [1,2]. It has a round or oval nucleus with clumped chromatin and, in one-half of cases, has a small but distinct nucleolus. The cytoplasm is usually moderate in amount and basophilic, but it may be scant in a minority of cases. The characteristic feature of villous lymphocytes, however, is the presence of thin and short cytoplasmic villi with uneven or sometimes polar distribution. The above features of villous lymphocytes are in contrast to typical hairy cells, which have numerous evenly distributed long, slender cytoplasmic projections, more dispersed chromatin pattern, larger cell size, and lower nuclear cytoplasmic ratio than that of villous lymphocytes. Nevertheless, the morphology of HCL variant and SLVL can be very similar [11].

In morphologically equivocal cases, surface marker studies used to be considered the final resort for a definitive diagnosis. The characteristic positive reactions to CD22, CD11c, CD25, and tartrate-resistant acid phospha-

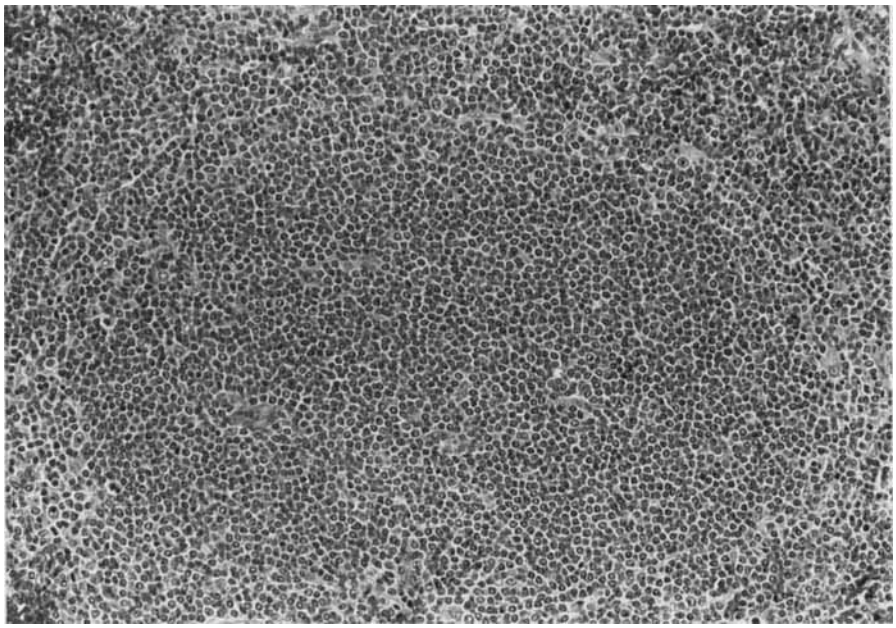

Fig. 6. Splenectomy specimen showing expansion of the white pulp by neoplastic lymphoid cells. (Hematoxylin & eosin stain, ×200.)

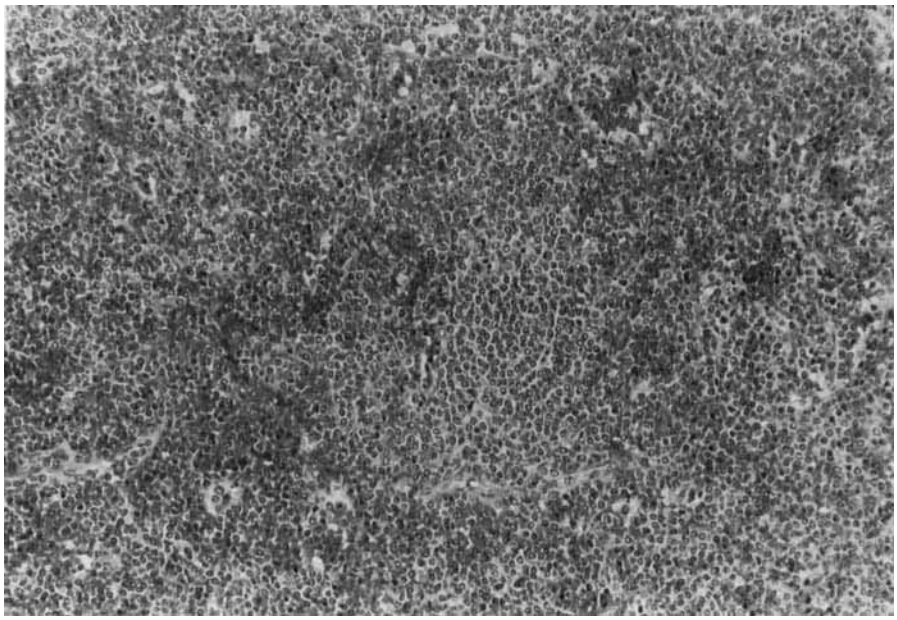

Fig. 7. Splenectomy specimen showing neoplastic infiltration of red pulp cords and sinuses. (Hematoxylin & eosin stain, ×200.)

tase staining were considered very specific for HCL. However, it was soon found that SLVL shared all the markers with HCL except CD25 [12]. The recent study by Matutes et al. [7] showed that even CD25 was positive in 25% cases of SLVL. Although Matutes et al. [7] stated that HC2 and B-ly-7 were most useful in distinguishing SLVL from HCL, these two monoclonal antibodies (MAbs) also reacted to less than 20% of SLVL cases. In fact, a relatively recent study by Posnett et al. [13], who produced the HC2 antibody originally, indicated that HC2 is an activation antigen of several hematopoietic cell lineage, inducible on monocytes by interferon-γ (IFN-γ). It appears that HC2 is also not so specific for HCL.

Since histologic studies showed that the tumor cells of

TABLE I. Immunophenotyping by Flow Cytometry (% Markers)

Antibody used	Cluster designation	Cell/antigen identified	BM (9/90)	PB (6/92)	BM (2/94)	SP (5/94)
Leu4	CD3	TCR complex	15	22	10	16
Leu1	CD5	T-cell, B-CLL	97	95	96	97
Leu9	CD7	T-cell	15	22	14	15
CALLA	CD10	Common ALL antigen	—	—	—	1
LeuM5	CD11c	Monocyte	81	67	80	78
My4	CD14	Myeloid cell	7	4	3	—
Leu12	CD19	B-cell	82	73	86	80
Leu16	CD20	B-cell	86	68	86	81
Leu14	CD22	B-cell	79	73	83	84
IL-2	CD25	IL-2 receptor	—	75	1	0
Hle	CD45	Pan-leukocyte	—	—	100	95
HLA-DR	—	B, activated T, myeloid	77	60	87	87
FMC-7	—	Prolymphocyte[a]	—	—	87	65
PV Ig	—	B cell	83	—	—	80
IgG	—	B cell	1	—	—	1
IgA	—	B cell	1	—	—	1
IgM	—	B cell	80	–	–	78
κ	—	B cell	83	76	78	84
λ	—	B cell	2	0	2	0

BM, bone marrow; PB, peripheral blood; SP, spleen; PV Ig, polyvalent immunoglobulin.
[a]FMC-7 may react with hairy cells but is negative for chronic lymphocytic leukemia cells. The high percentage of FMC-7-positive cells matched the percentage of prolymphocytes identified morphologically.

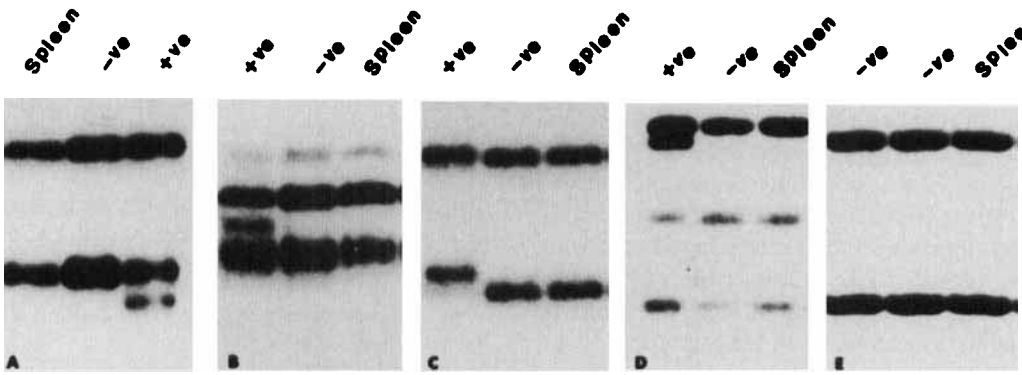

Fig. 8. SSCP analysis of p53 gene exon 5 (A), exon 6 (B), exon 7 (C), exon 8 (D), and exon 9 (E) in the DNA from spleen of the patient. No change in the mobility of DNA fragments was observed in these exons. −ve, control DNA from the peripheral blood of donor; +ve, DNA from a lymphoma tumor known to carry a mutation in the defined exon of the p53 gene.

SLVL may infiltrate the white pulp or the red pulp, or both, and the tumor cells can be small cells, large cells, or mixed small and large cells [3–6] and, because morphologic and marker studies are sometimes difficult, if not impossible, to distinguish SLVL from HCL variants, it seems most likely that SLVL represents a heterogeneous group of lymphoproliferative disorders, including at least some cases of HCL variants. In fact, both SLVL and HCL may be derived from the same origin, the splenic marginal zone [14,15].

HCL variant was originally described in a case with cytologic features intermediate between HCL and PLL and with a surface immunoglobulin of IgG [16]. However, the scope of HCL variant has now expanded to include hybrid forms of HCL/PLL and HCL/CLL, HCL blastic form, and HCL multilobular form [17,18]. When the histologic features are not typical for HCL, these HCL variant cases may then be classified as SLVL. For instance, some cases of HCL variant in the series reported by Sainati et al. [19] and one case of small lymphocytic lymphoma with circulating villous lymphocytes [20] may well be classified as SLVL.

In the present case, the phenotype was first consistent with a hybrid form of HCL/CLL, gradually changed into a hybrid form of HCL/PLL, and finally became predominantly PLL with negative conversion of CD25 and tartrate-resistant acid phosphatase. The morphology of

lymphoid cells in the peripheral blood corresponded to the phenotypic changes by showing predominantly small lymphocytes admixed with hairy cells and villous lymphocytes, gradually changed into predominantly prolymphocytes admixed with hairy cells and villous lymphocytes, and finally predominantly prolymphocytes with decreased numbers of hairy cells and villous lymphocytes.

Activation of oncogenes (e.g., c-*myc* and *bcl*-2), or mutation of tumor suppressor genes (e.g., p53), frequently occurs in lymphomas transforming to a higher-grade malignancy [14–21]. The absence of evidence for activation of c-*myc* and *bcl*-2, as well as mutation of p53 in our case, suggests that the evolution between HCL, CLL, SLVL, and PLL may represent a process triggered by unknown internal event within a cytogenetically homogeneous group of lymphoid neoplasms. Indeed, the presence of only one single rearranged band for both heavy chain and light chain genes substantiates our assumption that no genetic change has taken place during the evolution process.

By the current definition, this case can be diagnosed as SLVL, and it clearly shows that SLVL may include various lymphoproliferative disorders, such as HCL, CLL, PLL, or hybrid forms of different combinations. Melo et al. [1] suggested that HCL, HCL variant, SLVL, and PLL represent a spectrum of cell types frozen at slightly different stages during late B-cell maturation. In fact, these different cell types (e.g., CLL and PLL) can be induced by phorbol ester and evolve into other cell type (e.g., HCL) [22,23]. The present case suggests that evolution between SLVL and other low-grade B-cell lymphoproliferative disorders may take place as well. Therefore, SLVL appears to represent a broad spectrum, including HCL variant, CLL variant, PLL variant, and other subtypes, and the treatment of SLVL should be appropriately adjusted according to the subtypes.

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